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Plasmid Probes for Screening Specific Genes in Large Fragment DNA Libraries

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Heliothis virescens, a member of the lepidopteran family, is of great importance to many people who rely on agriculture as a source of income because it is a pest of many important crops. Because *H. virescens* feeds on a broad array of hosts including cotton, tobacco, tomato, and soybean it has earned its place as a major nuisance in the United States. *Heliothis subflexa* is a close taxonomic relative of *H. virescens*, but the two species differ in that *H. subflexa* is a specialized herbivore that only feeds on plants in the genus Physalis. Studies have been performed to determine whether a single locus on a gene is responsible for controlling the ability of *H. virescens* to feed on a wide array of hosts, or whether each individual host that *H. virescens* feeds upon is controlled by a separate locus. By determining what locus or loci on the gene controls *H. virescens'* ability to feed upon crops of agricultural importance there is a possibility to altering the gene and creating *H. virescens* that does not feed upon tobacco, cotton, soybean, and tomatoes. *H. virescens* has also been the subject of many studies regarding insecticide resistance, specifically *Bacillus thuringiensis* (Bt) toxin. The rise of insecticide resistance in agricultural pests is an example where a few major genes are consistently found to change in response to toxic chemical and biological agents. Bacterial Artificial Chromosome (BAC) libraries for *H. virescens* are important in providing a critical genomic resource to facilitate further investigations about Bt resistance. Actually identifying ecologically important genes of *H. virescens* requires fine scale mapping. Once the mapping has been completed, BAC libraries will be essential for isolating and sequencing candidate genes via positional cloning strategies.

The purpose of this project was to evaluate the true genome coverage of the lepidopteran BAC libraries by hybridizing a set of single copy, conserved nuclear DNA sequences from *H. virescens* to filters from the newly constructed libraries. The BAC libraries were constructed for several lepidopteran species, including *H. virescens* and *H. subflexa*, in order to advance research into genetic mechanisms related to lepidopteran development, behavior, morphology, and evolution. In the future the isolated genes will be used by other investigators to study their structure and how they are regulated.

The process for isolating probes from the plasmids began with bacterial transformation in order to introduce the DNA plasmid into bacterial cells. Next, the plasmids were isolated using a commercial kit. Once the plasmids were isolated, characterization of the plasmid was performed using agarose gel electrophoresis. This stage of the process confirmed the insert size of the plasmid was as expected. Then the plasmid was digested with restriction enzymes and purified. Finally, polymerase chain reaction (PCR) was used to amplify the inserts, which are composed of gene specific DNA, so they could be used to screen the BAC libraries.

I. Background

Heliothis virescens, a member of the lepidopteran family, is of great importance to many people who rely on agriculture as a source of income because it is a pest of many important crops. Because *H. virescens* feeds on a broad array of hosts including cotton, tobacco, tomato, and soybean it has earned its place as a major nuisance in the United States. *Heliothis subflexa* is a close taxonomic relative of *H. virescens*, but the two species differ in that *H. subflexa* is a specialized herbivore that only feeds on plants in the genus Physalis. Studies have been performed to determine whether a single locus on a gene is responsible for controlling the ability of *H. virescens* to feed on a wide array of hosts, or whether each individual host that *H. virescens* feeds upon is controlled by a separate locus. By determining what locus or loci on the gene controls *H. virescens'* ability to feed upon crops of agricultural importance there is a possibility to altering the gene and creating *H. virescens* that does not feed upon tobacco, cotton, soybean, and tomatoes. Previous studies have speculated that the difference in the diet of the two similar species, *H. virescens* and *H. subflexa*, is due to either a major mutation or from minor genetic changes (Sheck and Gould, 1996). With further research on the various genes of interest of *H. virescens*, people will be able to obtain a better understanding as to the location of these genes and this will make it easier to study methods to eliminate the role of *H. virescens* as an agricultural pest.

H. virescens has also been under many studies regarding insecticide resistance. The rise of insecticide resistance in agricultural pests is an example where a few major genes are consistently found to change in response to toxic chemical and biological agents. Resistance mechanisms in *H. virescens* in response to pyrethroids show point

mutations in a sodium channel. By using a genetic mapping strategy, it was shown that a lab strain of *H. virescens*, which shows a complete loss of binding to a *Bacillus thuringiensis* (Bt) toxin, developed resistance resulting from a cadherin-super family gene by retrotransposon-mediated insertion (Gill, Adang, and Gould). These examples are important because they reveal different classes of mutations in response to different insecticides. Bacterial Artificial Chromosome (BAC) libraries for *H. virescens* will be important in providing a critical genomic resource to facilitate further investigations about Bt resistance (Gill, Adang, and Gould). Actually identifying ecologically important genes of *H. virescens* will require fine scale mapping. Once the mapping has been completed, BAC libraries will be essential for isolating and sequencing candidate genes via positional cloning strategies.

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plasmid was digested with restriction enzymes and purified. Finally, polymerase chain reaction (PCR) was used to amplify the inserts, which are composed of gene specific DNA, so they could be used to screen the BAC libraries.

II. Materials and Methods

➔Source of plasmid DNA

Cloned *H. virescens* cDNA plasmids were sent to the lab for use with this project on filter paper. The cDNA inserts were derived from mRNA. In order to extract the DNA, a sterile razor was used to cut the filter paper into approximately 20 small pieces which were placed in a 1.5 ml microcentrifuge tube with 70 ul TE Buffer pH 7.0 (10 mM Tris adjusted to pH 7.0 with HCl, 1 mM EDTA). The tubes were then placed in a heating block at 60 ºC for approximately one hour. The tubes were then stored in the freezer at –20ºC for future use.

H. virescens cDNA plasmids for single copy genes (Table 1) were a generous gift of R. Palli (U. of Kentucky) and had been inserted into a plasmid vector, pBluescript SK (-) (Stratagene: ZAP-cDNA® Synthesis Kit) between the EcoRI and XhoI restriction sites (Figure 1). A plasmid is a small (3-5 Kb) DNA fragment that is circular. It contains a bacterial origin of replication, an ampicillin resistance region and a cloning site, which contains several restriction sites. The gene of interest is inserted into the plasmid vector.

Table 1: *H. virescens* cDNA plasmids for single copy genes

Provisional Gene Identification	Nucleotide Length of Sequenced Region
K386_ribosomal protein S4	621 nt
K713_Pumilio CG9755-PE	583 nt
J825_kisir	541 nt
K083_nucleoplasmin	1075 nt
K390_mRNA cap binding protein	814 nt
K555_lark CG8597-PA	560 nt
J819_probably p450	561 nt
L086_TIA-1 protein	574 nt
K405_eIf-4A	598 nt
K395_FK506 binding protein	533 nt
K396_vacuolar ATPase	685 nt

➔Bacterial Transformation

DH5α bacterial cells (Invitrogen) were thawed on wet ice. The required number of sterile 1.5 ml microcentrifuge tubes was placed on wet ice. A pipet was used to aliquot 50 ul of cells into each microcentrifuge tube. 1 ul of each sample of H. virescens cDNA that had been extracted from the filter paper was pipetted into a microcentrifuge tube containing 50 ul of DH5α cells. The competent cells and the cDNA were mixed by gently tapping the tube. The microcentrifuge tubes containing the competent cells and cDNA were incubated on wet ice for 30 minutes. The tubes were then heat-shocked for exactly 20 seconds in a 37ºC water bath and then placed on wet ice for 2 minutes. The contents of each microcentrifuge tube were added to a tube containing 950 ul of pre-

warmed LB medium (Per liter of LB medium add 5 g bactotryptone, 2.5 g bactoyeast extract, 5 g NaCl). The tubes were placed in a shaker at 37ºC for 60 minutes at 225 rpm. Once the tubes were finished shaking, 100 ul were plated onto LB agar plates containing 100 ug/ml ampicillin). By adding ampicillin to the medium it will allow the transformed cells to grow because the plasmid vector taken up by the cell contains a site of ampicillin resistance, but will inhibit any other unwanted growth. The plates were left on the lab bench for an hour to allow the transformation reaction to absorb into the LB agar. The plates were then inverted and incubated at 37ºC overnight.

➔Preparing Overnight Cultures

The next day each colony that had grown on the plates represented a clone, which contained millions of copies of the exact same plasmid. Overnight cultures were setup by sampling individual colonies from the plate and using them to inoculate individual tubes containing 5.0 ml LB broth and 5.0 ul ampicillin (50 mg/ml). The tubes were placed in a shaker at 37ºC overnight. The next day, the overnight cultures were observed for any evidence of growth. 1.5 ml of the bacterial culture were placed in a 2 ml microcentrifuge tube and centrifuged for 10 minutes in order to pellet the bacterial cells for use with a plasmid miniprep procedure.

➔Mini Prepping the Plasmids

The Perfectprep® Plasmid Mini Kit (Eppendorf) was used to obtain the plasmid DNA so it could be used in downstream applications. The lysate was decanted from the tubes containing pelleted bacterial cells using a pipet. The cells were then prepared according to the manufacturer's protocol enclosed with the kit (Appendix 1). Once the plasmid DNA was removed from the bacterial cells it was stored at -20ºC for use in other

procedures. The DNA was run on a 1% agarose gel in 0.5X TBE buffer to determine the yield of DNA and the insert size after staining with ethidium bromide (Figure 2).

➔Restriction Digest and PCR

PCR (D. Proestou, personal communication) and restriction digests were used in standard procedures to further analyze the DNA and create a probe to use for hybridization to *H. virescens* BAC library filters.

➔Restriction Digests

Quantification of the plasmid DNA obtained from the miniprep procedure revealed the following concentrations: K386 - 196 ng/ul and L086 - 344ng/ul. These samples were used to run a restriction digest using EcoRI and XhoI restriction enzymes and an EcoRI buffer (10X). The samples were made to be 50 ul in volume. Since L086 had a greater concentration than K386, only 3 ul of L086 DNA was added along with 2 ul of molecular grade water and 5 ul of K386 DNA was used. Each sample contained 1 ul of EcoRI restriction enzyme, 1 ul of XhoI restriction enzyme, 5 ul of EcoRI buffer, 0.5 ul of BSA (100X), and 37.5 ul of water. Once the samples were mixed, they were placed in a heating block at 37ºC for 4-5 hours.

➔PCR

The plasmid DNA obtained from the miniprep procedure was also used in a PCR reaction. The PCR kit used was an Epicenter kit and T7 (forward) and T3 (reverse) primers were employed. The PCR program utilized for this reaction was RCF-5, which included 30 cycles. The program ran in the following order: 1)Temp: 94 Time: 2 min 2)Temp: 92 Time: 30'' 3)Temp: 55 Time: 30'' 4)Temp: 72 Time: 90'' 5)Temp: 72 Time: 5 min. It was unclear whether this program would work for the samples being used

because the amplicon could have been too large, which meant the elongation time and number of cycles would have to be modified.

➔Filter Hybridization Protocol

The filters being used were *Bombyx mori*filters (*B. mori* R1 plates 5-8), which were generous gifts from C. Wu and H. Zhang of Texas A&M University. Since the filters had previously been used, they were washed with distilled deionized water on a shaker at 0.5 rpm for 5 minutes. The rinse was repeated in new water for 10 minutes. The filters were then placed in hybridization tubes with 20 ml of hybridization buffer (blocking reagent, NaCl, and pre-made buffer solution) and pre-hybridized for one hour at 42ºC.

The probe was created by adding 40 ng (2 ul) of K386 PCR product and 20 ng (4 ul) of K386 restriction digest product, which gave a total of 60 ng of DNA in 6 ul and a final concentration of 10ng/ul of DNA. Each filter required 30 ng of DNA and two filters were used, which required a total of 60 ng of DNA. The reason for mixing the PCR product and the digest product was because if just the digest product were used I feared I would not see any background, but if only the PCR product were used there could have been too much background since the T3 and T7 primers used created a product that contained a lot of additional vector sequence. The DNA was denatured at 95ºC for 5 minutes. After being denatured, the sample was placed on ice immediately for 5 minutes. The volume of the labeling reagent must equal the volume of the DNA being labeled, which meant that 60 ng of DNA in 6 ul would require 6 ul of labeling reagent (Amersham Biosciences). After adding the 6 ul of labeling reagent to the sample it was mixed and then 6 ul of gluteraldehyde (Amersham Biosciences) was added to the sample.

The sample was mixed and centrifuged briefly and then placed in the heating block at 37ºC for 10 minutes. The sample was then added to 1 ml of the hybridization buffer that had been pre-hybridizing with the filters and this mixture of probe and hybridization buffer was added to the hybridization tube containing the filters. The tube was placed back in the rotator at 42ºC to hybridize overnight.

After the filters had hybridized overnight, stringency washes were performed. The washing buffers were preheated to 55 ºC. After pouring off the hybridization buffer, 50 ml of Primary Washing Buffer #1 was added to the hybridization tube. The tube was placed in the hybridization over at 55 ºC for 10 minutes. The wash was then discarded and 50 ml of Primary Washing Buffer #2 was added to the tube. The tube was placed in the hybridization over at 55 ºC for 10 minutes. The wash was discarded and the filters were transferred to a shallow container containing Secondary Washing Buffer (2X SSC) at room temperature for 5 minutes. The wash was discarded and repeated for 10 minutes. Once the washes were completed, the signal detection procedure was performed.

III. Results

I used *H. virescens* plasmids K390 and K083 and a pUC19 control plasmid to perform my first transformations. After plating the transformation on an LB plate containing ampicillin and letting it incubate at 37ºC overnight, I obtained growth from both cDNA samples. I used colonies from each plate to setup overnight cultures. There was no growth in the overnight cultures, which could have been because I did not inoculate the overnight culture with enough cells from the colony on the plates, or the LB medium grown overnight could have been contaminated. Having decided to make new

LB medium and rerun overnight cultures, this time I used more cells to inoculate the overnight culture. The overnight cultures produced growth for both K390 and K083. I miniprepped both samples and ran the DNA on a gel using λ–HindIII digest standard as a marker. The gel revealed that the transformation must not have worked because there was not any detectable DNA on the gel (Figure 2).

I decided to rerun overnight cultures of K390 and K083 from colonies on the original transformed plates and then miniprep the transformed cells that grew in the overnight culture. This time when I ran the samples on a gel I was able to detect the presence of some stained DNA on the gel, but it was smeared and the stain was difficult to detect (Figure 3). This could have been because the gel was transferred to a new electrophoresis bath because I thought the original device was not working, which could have resulted in the loss of some DNA in the process.

Since the gel revealed low yields of DNA from the transformed colonies, it seemed logical to run additional transformations using K390 and K083 plasmid DNA, and also new samples, K386 and L086. The transformation did not reveal any growth on the plate containing K083, but growth was observed on the plates containing K386, K390, and L086. Overnight cultures for these three plasmids produced growth, which was then miniprepped using a miniprep kit (Eppendorf Perfectprep® Plasmid Mini) and a coworker (D. Proestou) prepped the samples using an Alkali-lysis protocol. The DNA obtained from the miniprep kit and the alkali-lysis procedure was used to run an agarose gel which revealed the insert sizes for K390, K386, and L086 to be approximately 1.4 kb, 1.0 kb, and 1.5 kb respectively, based on the λ–HindIII size standard. The samples

prepared using the miniprep kit seemed to produce slightly higher yields than the alkali-lysis procedure (Figure 4).

The results from the gel revealed that DNA was in fact present, so the next step was to run a restriction digest on the samples. After running the restriction digests using EcoRI and XhoI restriction enzymes it was clear that the concentration of DNA contained in the gel bands would not be large enough to use to create probes for hybridization, which required approximately 10 ng/ml, and 30 ng/filter (Figure 5). An additional restriction digest was performed using increased amounts of DNA in the hope of obtaining a concentration of 10ng/ul after performing the gel clean-up system on the gel band. It made sense to only use K386 and L086 for further procedures, and discontinue usage of K390 because the concentration was so low for this sample that it would be difficult to obtain the concentration required for further applications.

Unfortunately, despite loading approximately twice the amount of DNA from the earlier attempt, the K386 and L086 bands on the gel did not look much more concentrated (Figure 6). After removing the gel bands and cleaning them using the Eppendorf kit, I quantified the DNA I had obtained for K386 and L086. Both samples had a lower concentration than needed to create a probe (K386: 3.78 ng/ul and L086: 4.706 ng/ul), but K386 could potentially be used if it was ethanol precipitated to increase the concentration.

In order to learn how filter hybridization is performed, I used the restriction digest product from K386 that had been cleaned up and ethanol precipitated together with the PCR product that D. Proestou had obtained. Since it was unclear whether the probe for K386 would actually work, I did not use *H. virescens* filters because I did not want to

waste them on a hybridization that might not work. Instead, I used filters from a *Bombyx mori* library.

When preparing the probe to run the hybridization, restriction digest product was mixed with PCR product. Because gene of interest was inserted between the restriction sites EcoRI and XhoI, and the plasmid was cut at EcoRI and XhoI, the restriction product only contained the insert so most likely would not produce any background with the *B. mori* filters unless there were a positive clone containing the same gene. On the other hand, the PCR product was formed using T3 and T7 primers, which means it contained approximately 40 nucleotides worth of vector sequence from the T7 primer binding site and 90 nucleotides from the T3 primer binding site (Figure 1) in addition to the insert. The presence of the additional vector DNA increases the chances of obtaining background signals with the *B. mori* filter, acting as a positive control for the overall hybridization. After performing the hybridization using two filters from the *B. mori* library and developing the film, there was no background nor were there any hits.

IV. Discussion

I was not expecting any hits because the filters I used were from a different species than the probe. The fact that there was no background could be due to a number of reasons. One might have to do with the preparation of the probe. It was difficult to obtain a sample with a high enough DNA concentration to perform a hybridization. Also, since the probe was a mixture of PCR and restriction digest products this could have resulted in an inefficient probe. A higher amount of PCR product was used than restriction digest product, which could have prevented the hybridization from giving a

strong enough signal to detect. Another reason for no detection of background could be due to methodological problems in performing the hybridization procedure, such as improper preparation of hybridization buffers. An additional reason why background might not have been detected could be due to differences in the vectors. The vector containing the *B. mori* DNA could be different from the plasmid vector containing the *H. virescens* DNA, which would mean that no image would be present upon developing the film. Since the source of the *B. mori* filters was known, I was able to lookup the vector that was used. According to the Texas A&M website, which is where C. Wu and H. Zhang sent the *B. mori*filters from, the vector is pBeloBAC11 and PECBAC1 (Wu, C and H. Zhang). These vectors are larger than the pBluescript SK (-) that contains the H. virescens cDNA. The restriction sites also appear to differ from one another.

While the hybridization did not reveal any positive signals, this does not mean that the experiment was unsuccessful. The probe that was created using DNA from K386, thought to code for a ribosomal protein in *H. virescens*, could actually work, but maybe it did not hybridize to *B. mori*DNA even though it is a well -conserved gene at the amino acid level because they are different species which may have different sequences at the DNA level. Any studies that are performed to analyze genes of interest in *H. virescens* are important in further investigating their role as an agricultural pest, as well as their resistance to toxins. The more that is known about the function and location of each gene and its surrounding or regulatory DNA, researchers will be better able to determine why *H. virescens* has such a broad host range, whereas its close relative *H. subflexa* does not. If this can be determined, perhaps the species can be crossed and the genetic component

that controls *H. virescens*' ability to feed on soybean, cotton, tobacco, and tomato can be eliminated. This could reduce and eventually eliminate *H. virescens* as a crop pest.

Insecticide resistance is also becoming a problem with *H. virescens*, but careful research into the genetic components that create this resistance within the species can lead the way for developing new methods to control the species. It may be that insecticides are not the best control method for this pest because they have the ability to develop resistance to toxins relatively quickly. If researchers could determine what genes are responsible for creating the mutations that cause resistance to certain toxins, more efficient control strategies could be initiated, which would greatly benefit people who rely on agriculture as a primary source of income as well as make production of our food supply more environmentally friendly and more efficient.

This semester long project provided me with a wonderful opportunity to work in a laboratory on a daily basis, as well as carry out procedures that I would never have had the opportunity to experience. Not only did I learn basic lab skills, but also the problem solving and troubleshooting that arise with any research project. The lessons and knowledge that I have gained through this project will continue to assist me in many aspects of my future in veterinary school and eventually as a practicing veterinarian.

References

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Sheck, A. L., and F. Gould. 1996. The genetic basis of differences in growth and behavior of specialist and generalist herbivore species: selection on hybrids of *Heliothis virescens* and *Heliothis subflexa* (Lepidoptera). Evolution 50: 831 -841.

Palli, R. *H. virescens* cDNA plasmids for single copy genes. University of Kentucky.

Wu, C. and H. Zhang. Texas A&M BAC Libraries. GENEfinder Genomics Resources. Texas A&M University. Available WWW: http://hbz.tamu.edu/cgi-bin/htmlassembly?bacs.

Appendix 1

Perfectprep® Plasmid Mini Protocol (Eppendorf)

1. Transfer up to 1.5 ml of bacterial culture to a microcentrifuge tube and centrifuge at 12,000 - 16,000 x g for 20 seconds to pellet cells.

2. Decant or aspirate supernatant, making sure not to disturb the bacterial pellet.
Note: If desired, add another 1.5 ml of culture to the pelleted bacteria in step 2 and repeat steps 1 and 2. **Do not process more than 3 ml of culture per isolation.**

3. Add 100 µl Solution 1 to the pellet and completely resuspend cells by vigorous vortexing.

4. Add 100 µl Solution 2 and mix well by repeated gentle inversion.

5. Add 100 µl Solution 3 and mix well by repeated vigorous inversion.

6. Centrifuge at 12,000 - 16,000 x g for 30 seconds and transfer supernatant to a Spin Column in a Collection Tube.

7. Vigorously and thoroughly mix the DNA Binding Matrix suspension before pipetting.

8. Add 450 µl DNA Binding Matrix to the Spin Column and mix.

9. Centrifuge the Spin Column/Collection Tube assembly at 12,000 - 16,000 x g for 30 seconds. Decant filtrate and place Spin Column back into the Collection Tube.

10. Add 400 µl DILUTED Purification Solution to the Spin Column and shake briefly. Centrifuge at 12,000 - 16,000 x g for 60 seconds.

11. Decant filtrate and place Spin Column back in the Collection Tube. Centrifuge at 12,000 - 16,000 x g for 60 seconds.

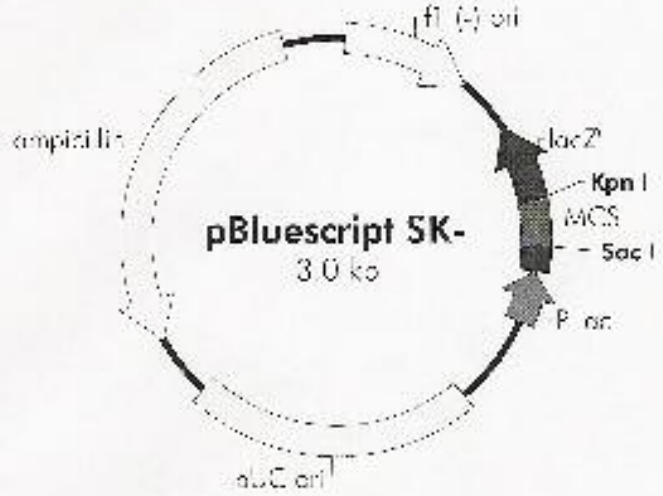
12. Transfer Spin Column to a fresh Collection Tube and add 50 to 70 µl of Elution Buffer
(or Molecular Biology Grade Water) to the DNA Binding Matrix. Vortex briefly.

13. Centrifuge at 12,000 - 16,000 x g for 60 seconds.

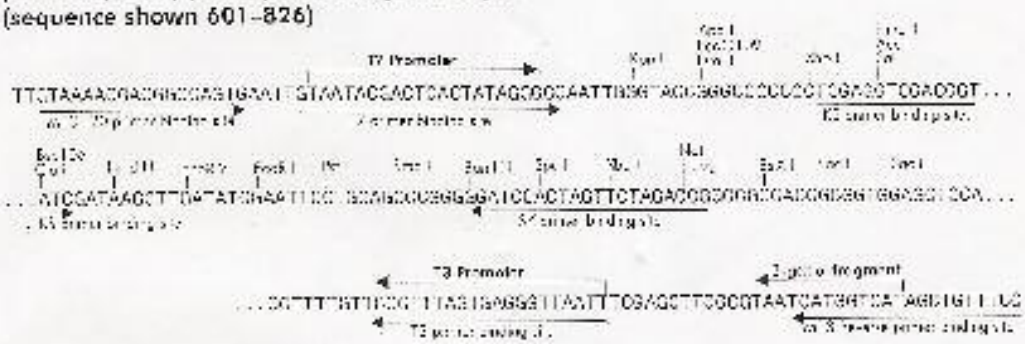
14. Discard the Spin Column and cap the Collection Tube. The eluted plasmid DNA is now ready for use in downstream applications or can be stored at either 4°C or -20°C for future use.

Figure 1:

pBluescript® SK(–) Vector Map



pBluescript SK (–) Multiple Cloning Site Region
(sequence shown 601–826)



Feature	Nucleotide Position
f1 (–) origin of ssDNA replication	21–307
3′ splice junction of fragment coding sequence (lacZ)	469–616
T7 promoter/transcription initiation site	648
multiple cloning site	676–770
T3 promoter/transcription initiation site	774
[illegible]	811–959
pUC origin of replication	1158–1825
ampicillin resistance (bla) ORF	1974–2834

FIGURE 3 Circular map and polylinker sequence of the pBluescript SK(–) plasmid. The complete sequence and list of restriction sites are available from www.stratagene.com or from the GenBank® database (X52324).

.1% agarose gel 0.5xTBE
- loaded 3 ul λ standard and 5 ul of each DNA sample
- added 0.5 ul loading dye to each sample
- ran for 30 min
- ethidium bromide stain

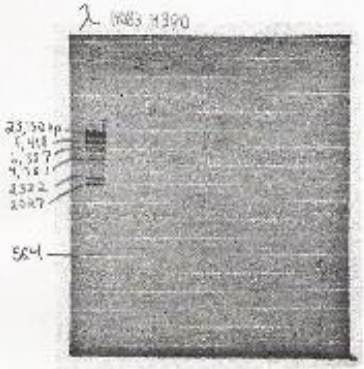


Figure 2: Plasmid DNA obtained from miniprep kit

- 1% agarose gel in 0.5X TBE buffer
- loaded 3 µl of rxn. DNA sample and 0.5 µl loading dye
- gel run for 30 min at 90 V
- stained in Ethidium Bromide

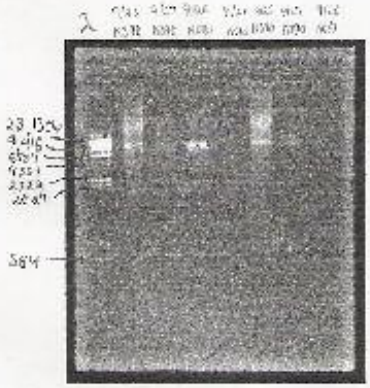


Figure 3: Plasmid DNA obtained from miniprep kit

Miniprep from 10/27
- 1% agarose gel
Ran 30 min at 150V
- loaded 5 µl (150ng) λ standard
- loaded 5 µl of each plasmid prep
- stained 1% Biomidium

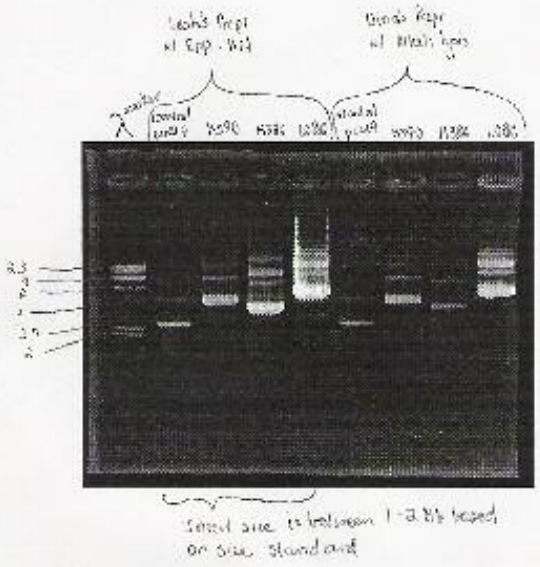


Figure 4: Plasmid DNA obtained from using miniprep kit and alkali-lysis procedure

Restriction Digest #1

- Added 10ul loading dye to 10ul restricted digest samples
- loaded 8 lanes of each sample w/ 10ul in each lane
- loaded 5ul molecular
- 1% agarose gel
- Ran for 35 min
- stained in EtBr for 18 min

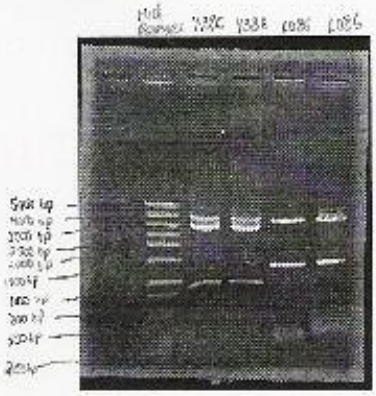


Figure 5: Results from Restriction Digest #1

Restriction Digest

Restriction Enzymes: EcoRI and XhoI
Restriction Buffer: EcoRI
Added 5 ul 1338 DNA
3 ul 1086 DNA + 2 ul water

- loaded 5 ul Midrange standard mixed with 20 ul loading dye
- 1338: loaded 25 ul sample and 5 ul loading dye
- 1086: loaded 30 ul of a 50 ul sample that had been mixed with 10 ul loading dye
- Ran on 1% agarose gel in 0.5x TBE buffer for 30 min.

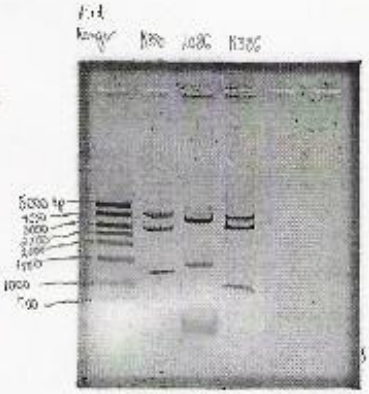


Figure 6: Results from Restriction Digest #2